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## Final Report

This represents the final report for this project. In this summary, we will describe what we think are the most important findings, the drug combination of greatest promise for further testing and additional work stimulated by this research.

## Agents Exhibiting Greatest Synergy

After reviewing all of the drug combinations tested, the chemotherapeutic agent that illustrated the most promising synergy with other drugs was taxol. In particular, the combination of taxol with the HMG-CoA reductase inhibitor, mevastatin, and with the differentiating agent, phenylbutyrate, showed the most significant synergy. However, the combination of taxol with combination of mevastatin and phenylbutyrate showed no increase in antitumor efficacy compared with taxol plus mevastatin or taxol plus phenylbutyrate. These results make sense based on what is known about the mechanism of action of taxol, mevastatin, and phenylbutyrate.

## Possible Mechanistic Basis for Synergy

Ras, rho and related proteins play a critical role in signal transduction events regulating cell growth and motility. These proteins typically must undergo post translational modification involving the addition of either farnesol- or geranylgeranyl- groups. This reaction involves a condensation reaction between the protein and farnesol pyrophosphate or geranylgeranyl pyrophosphate. In this reaction, the pyrophosphate acts as a leaving group and resulting addition of farnesol- or genanylgeranyl- to a cysteine on the protein.

The farnesol- and geranylgeranyl- groups are composed of isoprenyl- subunits. These isoprenyl subunits are synthesized from acetic acid by a pathway that starts with HMG-CoA reductase. Subsequent steps in the pathway include the formation of mevalonate, mevalonate 5-pyrophosphate and isopentenyl pyrophosphate. In addition to the formation of farnesyl pyrophosphate and geranylgeranyl pyrophosphate, isopentenyl pyrophosphate is an intermediate in the synthesis of a wide range of important molecules, including cholesterol, steroid hormones, and coenzyme Q10.

Mevastatin, phenylbutyrate and taxol all inhibit separate steps along the pathway leading from acetate to the farnesylation or geranylgeranylation of ras-like proteins. Mevastatin and other statin drugs block the formation of isopentenyl pyrophosphate by blocking the HMG-CoA reductase. Among its many biochemical actions, phenylbutyrate inhibits the synthesis of isopentenyl pyrophosphate from mevalonate pyrophosphate. Finally, we have previously shown that taxol inhibits the addition of these polyprenyl moieties to ras-family proteins[1]. Thus, a reasonable hypothesis for the synergy seen between taxol and mevastatin or phenylbutyrate is that the act together to inhibit posttranslational modification of ras-family proteins needed for prostate cancer cell survival. This hypothesis is supported by the observation that the farnesyltransferase inhibitor, manumycin, also interacts synergistically with taxol[2].

## Choice of Combinations for *In Vivo* Testing

The current grant did not cover *in vivo* testing of the most promising combinations. Nevertheless, enough is known about drug levels that are achievable *in vivo* to make recommendations. Statin drugs, including mevastatin, are all generally used for lowering cholesterol levels at concentrations between 1 and 50 nM. We previously conducted a phase 1 clinical trial of lovastatin and found blood levels of 100-500 nM are tolerated with modest side effects largely limited to muscle weakness[3]. This was associated with a marked decline in serum coenzyme Q10 levels and responded to oral replacement with 200 mg of coenzyme Q10. Unfortunately, in the studies covered under this grant and in earlier work, neither mevastatin nor lovastatin exhibit significant synergy with taxol at blood levels below 10 uM. As a result, further testing of taxol-statin drug combinations do not appear to be promising.

In phase 1 clinical trials testing intravenous phenybutyrate, we found that blood levels between 500 and 2,000 uM were well tolerated[4]. This is well in excess of the concentration required to significantly increase the antitumor activity of taxol. Furthermore, the oral bioavailability of phenylbutyrate is close to

50% and doses of 20 grams three times a day have been well tolerated[5] [6]. An additional advantage is that phenylbutyrate is an FDA approved drug for the treatment of children with genetic defects in the urea cycle. When used for these indications, chronic phenylbutyrate has proved to be remarkably well tolerated, even after years of constant administration. For these reasons, the combination of taxol and phenylbutyrate appears to be the most promising combination to emerge from the studies funded by this grant.

**Additional Work Stimulated By This Grant**

Taxol and taxotere have both proved to be active antiangiogenesis agents[7] [8]. These agents appear to act by suppressing the production of VEGF[9]. Additionally, tumor overexpression of VEGF renders cancer cells relatively resistant to the antiangiogenesis action of the taxanes. Other agents that reduce VEGF production, such as thalidomide or antiVEGF antibody, enhance the activity of taxanes[9] [8]. Relevant to the work done under this DOD grant, the farnesyltransferase inhibitor, manumycin, acts synergistically with taxol to block angiogenesis as well as tumor cell kill[2, 10]. This suggested to us the possibility that there might be a parallel between agents that synergistically enhance tumor cell kill by taxol and those that enhance it's ability to block angiogenesis.

We have been able to obtain a $400,000 per year grant from a drug company to pursue this line of research. Rather than focus on agents that broadly inhibit protein isoprenylation, we chose to identify what we think is the most important of the ras-family proteins for angiogenesis. In collaboration with Avril and Andrew Somlyo, we selected rho, because if its important role in endothelial cell invasiveness and tube formation. We have shown that an orally active inhibitor of rho action significantly suppresses endothelial cell invasiveness and tube formation[11]. *In vivo,* the combination of taxol and the rho inhibitor were more effective than either agent alone in suppressing the growth of the androgen-independent cell line, PC3. Marimastat is a metalloproteinase inhibitor that suppresses tumor cell invasiveness and angiogenesis. This agent is very well tolerated. A recent randomized controlled clinical trial found marimastat comparable to gemcitabine in the treatment of pancreatic carcinoma. If confirmed, this would make marimastat first line treatment for pancreatic carcinoma. We found that the three-drug combination of taxol, marimastat, and rho inhibitor resulted in dramatic suppression of PC3 *in vivo*, making this combination an attractive one to bring to clinical trial.

These results have led us to entertain the hypothesis that it will be possible to develop a taxol-based combination that synergistically enhances apoptosis and suppresses tumor angiogenesis.

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